J Musculoskelet Neuronal Interact 2007; 7(1):56-61

3rd Black Forest Forum
for Musculoskeletal Interactions,
May 4-7, 2006

Hylonome

Application of skinned single muscle fibres to determine myofilament function in ageing and disease

H. Degens[1] and L. Larsson[2]

[1]Institute for Clinical and Biophysical Research into Human Movement, Manchester Metropolitan University, Alsager Campus, Alsager, Cheshire, UK; [2]Department of Clinical Neurophysiology, Uppsala University, Uppsala, Sweden

Abstract

The chemically skinned fibre is a suitable preparation to determine whether alterations in myofilament function contribute to muscle dysfunction during ageing and disorders such as chronic obstructive pulmonary disease (COPD). In this preparation the sarcolemma is chemically permeabilized and the myofilament lattice kept intact, functioning under controlled near-physiological conditions. As force generating capacity is an important determinant of muscle function and is related to fibre cross-sectional area (FCSA), we compared several methods employed by researchers to determine FCSA. Specific tension, force divided by FCSA, has a co-efficient of variation of 27%, 37%, or 30% when the FCSA was measured from the width and depth assuming an elliptical circumference, the width assuming a circular circumference, and the width while the fibre was suspended in the air, respectively. The last method showed the closest relation with the FCSA in histological sections. The velocity of maximal unloaded shortening (V_0) varied with fibre type, with fibres expressing the β/slow (type I) myosin heavy chain (MyHC) isoform being the slowest and fibres expressing the IIb MyHC isoform the fastest. While muscle weakness experienced after surgery could not be explained by changes in specific tension or FCSA of individual fibres, the preparation revealed significant changes in myofilament function during ageing and COPD.

Keywords: Skinned Single Fibres, Specific Tension, Shortening Velocity, Ageing, COPD

Introduction

Muscle is a highly plastic tissue and shows morphological and functional adaptations to increased[1] and decreased use[2]. Ageing[3] and many chronic disorders, such as chronic obstructive pulmonary disease (COPD) and heart failure, are often accompanied by muscle dysfunction[2]. Although this is indicative for myopathic changes during these conditions, muscle dysfunction might also be attributable to disuse-related atrophy or other factors, such as those directly related to the disease, e.g. an impairment of full recruitment of the muscle to prevent dyspnoea during COPD. Therefore, it is important to determine whether muscle dysfunction is caused by alterations within the muscle itself or by other factors.

Muscle dysfunction is primarily reflected by muscle weakness and loss of power generating capacity. However, determination of muscle strength by voluntary isometric contractions does not necessarily reflect the quality of the individual muscle fibres. During ageing, for instance, the loss of force generating capacity can not be explained by an altered central drive, muscle atrophy or muscle fibre pennation alone and an altered single muscle fibre specific tension has been forwarded as an important underlying mechanism[4]. The role of the moment arm, elasticity of the tendon and the many fibres of different types that compose the muscle further compound *in vivo* muscle function measurements. Clearly, it is difficult to ascertain from *in vivo* determination of muscle contractile properties whether muscle dysfunction is solely attributable to muscle fibre atrophy, shifts in fibre type composition and/or to altered contractile

The authors have no conflict of interest.

Corresponding author: Dr. ir. Hans Degens, Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Alsager Campus, Alsager, Cheshire ST7 2HL, UK
E-mail: h.degens@mmu.ac.uk

Accepted 19 May 2006

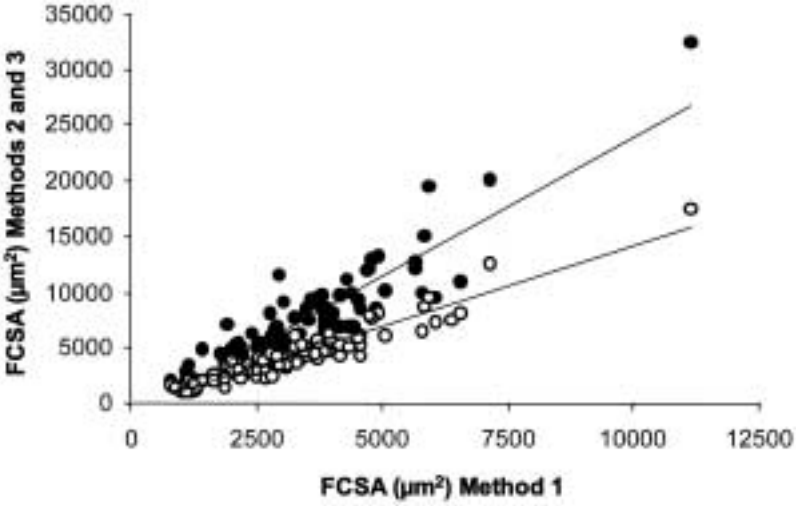

Figure 1. Comparison of different methods to calculate the fibre cross-sectional area (FCSA) of skinned single fibres; Method 1: from the width and the depth, assuming an elliptical circumference, Method 2: from the width, assuming a circular circumference, and Method 3: from the width while the fibre was suspended in the air. ●: Method 2 vs. Method 1, R=0.90 (n=119); ○: Method 3 vs. Method 1, R=0.95 (n=0.95). Method 3 vs. 2 R=0.95 (n=63).

properties of the individual myofibres. Skinned fibres circumvent these limitations and provide a powerful tool to determine the function of the myofilaments under controlled near-physiological conditions. In this preparation the sarcolemma is chemically permeabilized and the myofilament lattice kept intact. The skinned fibre preparation has frequently been used in experimental animal models. This method is also very useful in studies of regulation of contraction in single human fibres obtained from percutaneous muscle biopsy specimens. The usefulness of this preparation has been repeatedly documented in normal[5-10] and pathological muscle tissue[11-14].

In the remaining part of this paper we will briefly describe the chemically skinned fibre preparation and give some examples of the use of these fibres to answer questions related to muscle dysfunction during ageing, after abdominal surgery, and diaphragm weakness during COPD. The force generating capacity per fibre cross-sectional area (FCSA), specific tension, is an important determinant of muscle fibre function. Therefore, not only accurate measures of maximal muscle fibre force, but also of fibre cross-sectional area (FCSA) must be obtained. We will compare three methods that have been employed in the literature to determine FCSA: 1) from the measurement of the width and depth assuming an elliptical circumference[7,11,13-15]; 2) the width, assuming a circular circumference[16]; and 3) equating the width with the depth while the fibre is suspended in the air[17]. Finally, as power is a product of force and velocity we will also show that maximal unloaded shortening velocity (V_0) is largely determined by the myosin heavy chain (MyHC) and how V_0 is affected during ageing.

	Histology	Method 1	Method 2
Muscle 1	2040±470 (100)	2430±760 (15)	6730±1620 (15)
Muscle 2	3190±1020 (100)	4350±870 (8)	9940±2250 (8)
Data are mean ±SD, with number of fibres between parentheses.			

Table 1. Comparison of the fibre cross-sectional area (FCSA) determined in histological cross-sections of rat soleus muscles and single fibres of the same muscles by Method 1, calculating the FCSA from the depth and the width of the fibre, and by Method 2, calculating the FCSA from the width of the fibre assuming a circular circumference.

Methods

The procedure has been described previously[8,11,13-15,18]. A percutaneous biopsy provides sufficient material to isolate many single fibres. Briefly, the biopsy is collected in a relax solution (in mM: 4.5 MgATP, 1 free Mg^{2+}, 10 imidazole, 2 EGTA, 100 KCl, pH 7.0) on ice. Small bundles are separated and stretched to 110% slack length and subsequently transferred to 50% glycerol/relax solution at 4°C for skinning. After 24 hours the bundles will be transferred to -20°C until use. Immediately before use the bundles are placed in relax/0.5% brij-58 or relax/1% Triton X-100 for 20 minutes for permeabilization of the membranes. Then the fibres are attached to a motor arm and a force transducer (Aurora, Canada) on a platform cooled to 12°-15°C on an inverted microscope and suspended in relax solution. Sarcomere length is set at 2.4-2.8 µm, dependent on species. Before activation the fibre is transferred to a low-EGTA solu-

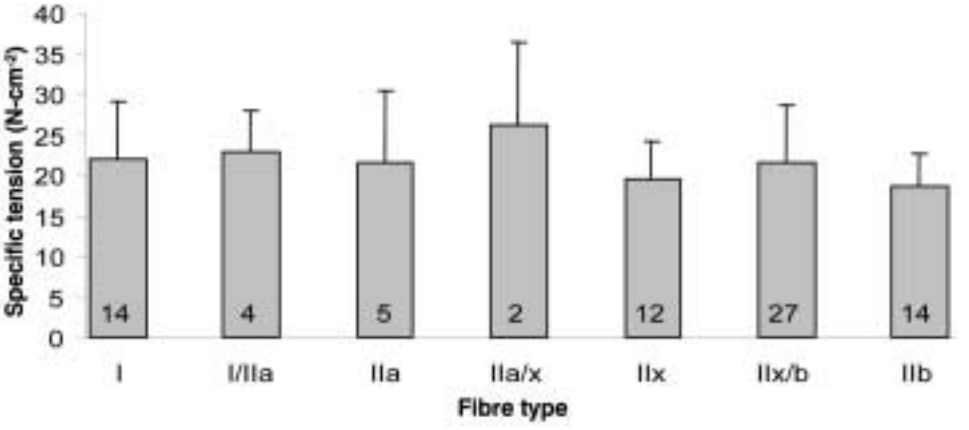

Figure 2. Specific tension in single skinned fibres of different type from rat muscles. On the x-axis indicated the myosin heavy chain isoform composition of the fibre. The number of fibres is indicated in the bars. Values are mean ±SD.

tion (same as relax except the EGTA is 0.5 mM instead of 2 mM) for 15 seconds[7]. Then the fibre is maximally activated with activating solution (in mM: 5.3 MgATP, 1 free Mg^{2+}, 20 imidazole, 7 EGTA and 19.6 creatine phosphate, 64 KCl, pH 7.0) containing a high [Ca^{2+}], i.e. pCa 4.5. The force recorded minus the resting tension is given as the maximal force (P_0). Dividing P_0 by the FCSA, corrected for 20% swelling that occurs during skinning, gives specific tension. Fibre width is measured as the shortest distance between the long sides of the fibre and the depth from the vertical displacement of the objective while focusing on the top and bottom of the fibre. FCSA is calculated from: 1) the width and depth, assuming an elliptical circumference; 2) the width assuming a circular circumference; and 3) from the width of the fibre when suspended in air assuming a circular circumference. For two muscles Method 1 and 2 were compared with the FCSA seen in a histological section stained for myosin ATPase after acid pre-incubation. From data in a previous paper[11] the comparison of Methods 1 and 3 with histological FCSA can be derived.

The V_0 is obtained with the slack test[8,15,18,19]. During maximal activation slacks of different amplitude are given. The slope of the slack amplitude and the time to take up the slack gives V_0.

The myosin heavy chain is the primary determinant of V_0[5] and also specific tension is thought to vary with fibre type[17]. Therefore, after the experiments the MyHC composition is determined by 7% SDS/PAGE with 30% glycerol in the separation gel for 27 hours, 120 V at 15°C as described previously[8,18].

Results and discussion

Comparison of methods to determine FCSA

There is a good correlation between the three methods to determine FCSA (Figure 1). They do, however, not give the same FCSA; the FCSA calculated from the width assuming a circular circumference (Method 2) being the largest, that derived from the depth and width of the fibre (Method 1) being the smallest and that calculated from the fibre suspended in the air (Method 3) in between. Table 1 shows that the FCSA determined with Method 1 is closer to that measured in a histological section than that determined with Method 2. In a previous study[11] we found that Method 3 tended to be even closer than Method 1 to the FCSA in histological sections. This suggests that Method 2 is not the best to obtain a realistic estimate of FCSA. Clearly, the way the FCSA is calculated affects the calculated specific tension and may explain at least part of the differences in specific tensions reported in the literature. In addition, to calculate specific tension we corrected the FSCA for 20% swelling[20], which other groups may not do. But even when taking into account this swelling Method 2 overestimates FCSA.

Specific tension was determined in 43 fibres using the FCSA obtained with each of the methods to assay the co-variance of specific tension. The co-variance was lowest for Method 1 (27%), highest for Method 2 (37%) and in between for Method 3 (30%). This and the fact that the specific tension calculated with the FCSA from Method 2 is less than 70% of that obtained by the other methods suggests that a fibre in solution is far from circular, and that the other two methods are preferable to determine changes in FCSA and specific tension.

Some authors have reported higher specific tensions for type II than type I fibres[17]. Here we observed, in line with previous observations[13], no significant difference in specific tension between fibre types (Figure 2). We found a clear relation between the MyHC composition of a fibre and V_0, i.e., I<IIa<IIx<IIb and intermediate V_0's in hybrid fibres co-expressing two MyHC isoforms (Figure 3).

Examples of application of single skinned fibres in ageing and disease

Ageing. Using skinned fibres to study the effects of ageing, a significant decline in V_0 in fibres expressing the type I

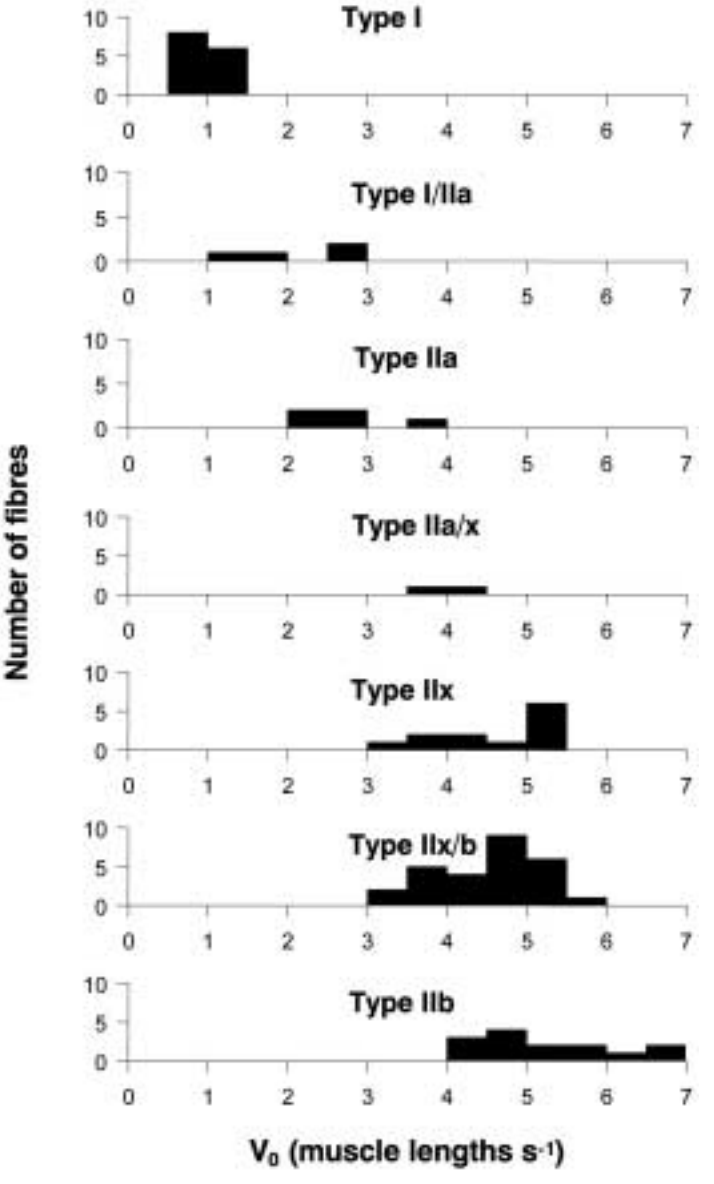

Figure 3. Unloaded shortening velocity (V_0) of single skinned rat muscle fibres of different type.

MyHC isoform in rats[18,21] and fibres expressing type I and IIa MyHC isoforms in humans[15,22] has been observed which was not explicable by changes in myosin heavy chain and light chain composition. This suggests that part of the decline in power generating capacity during ageing[23] is attributable to alterations in the myofilaments. Subsequent *in vitro* motility assay studies on myosin extracted from single muscle fibres expressing the type I MyHC isoform from mouse, rat and human skeletal muscle confirmed that alterations in myosin causes this decline in V_0[24,25]. Glycosylation of myosin results in similar changes in the *in vitro* motility speed of actin filaments over myosin coated slides[26] suggesting that glycosylation of myosin may cause the decline in V_0 during ageing.

Post-operative muscle weakness. Muscle weakness is often observed in patients during the post-operative period after major surgery. The post-operative increase in insulin resistance might result in increased muscle protein breakdown and decreased protein synthesis. It was therefore hypothesised that muscle weakness experienced by patients is caused by muscle wasting and possibly also by a reduction in the specific tension of muscle cells. To investigate this, muscle biopsies were obtained from the vastus lateralis muscles from patients before and 3-6 days after abdominal surgery. Despite the development of insulin resistance in all patients there was no evidence of muscle fibre atrophy or a decline in specific tension of individual muscle cells[11]. Thus the experienced post-operative muscle weakness is attributable to other factors than muscle wasting and changes in the myofilaments.

Diaphragm weakness during COPD. Muscle weakness and muscle wasting are frequently observed in a chronic condition such as COPD[2]. Not only peripheral muscles, but also the diaphragm is affected and respiratory failure, the result of diaphragm weakness, is the primary cause of death in patients with COPD[27]. Using single fibres, it was found that the specific tension and the Ca^{2+} sensitivity of diaphragm muscle cells were reduced. The decreased specific tension was attributed to a decline in the myosin content of the individual muscle cells, which was probably related to activation of the ubiquitin proteasome pathway[13].

Summary

In summary, the skinned single fibre preparation is a powerful tool to determine whether muscle dysfunction during ageing and disorders is related to alterations in the function of the myofilaments. Furthermore, sensitive electrophoretic techniques allow one to determine modifications of proteins and changes in protein expression that may underlie the myocyte dysfunction during those conditions.

References

1. Salmons S, Henriksson J. The adaptive response of skeletal muscle to increased use. Muscle Nerve 1981; 4:94-105.
2. Degens H, Alway SE. Control of muscle size during disuse, disease, and aging. Int J Sports Med 2006; 27:94-99.
3. Larsson L, Ansved T. Effects of ageing on the motor unit. Prog Neurobiol 1995; 45:397-458.
4. Morse CI, Thom JM, Reeves ND, Birch KM, Narici MV. *In vivo* physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. J Appl Physiol 2005; 99:1050-1055.
5. Bottinelli R, Betto R, Schiaffino S, Reggiani C. Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. J Physiol 1994; 478:341-349.
6. Frontera WR, Larsson L. Contractile studies of single human skeletal muscle fibers: a comparison of different muscles, permeabilization procedures, and storage techniques. Muscle Nerve 1997; 20:948-952.
7. Greaser ML, Moss RL, Reiser PJ. Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chains. J Physiol 1988; 406:85-98.
8. Larsson L, Moss RL. Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. J Physiol 1993; 472:595-614.
9. Larsson L, Salviati G. A technique for studies of the contractile apparatus in single human muscle fibre segments obtained by percutaneous biopsy. Acta Physiol Scand 1992; 146:485-495.
10. Stienen GJ, Kiers JL, Bottinelli R, Reggiani C. Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. J Physiol 1996; 493(Pt2):299-307.
11. Degens H, Soop M, Hook P, Ljungqvist O, Larsson L. Post-operative effects on insulin resistance and specific tension of single human skeletal muscle fibres. Clin Sci (Lond) 1999; 97:449-455.
12. Larsson L, Li X, Tollback A, Grimby L. Contractile properties in single muscle fibres from chronically overused motor units in relation to motoneuron firing properties in prior polio patients. J Neurol Sci 1995; 132:182-192.
13. Ottenheijm CA, Heunks LM, Sieck GC, Zhan WZ, Jansen SM, Degens H, de Boo T, Dekhuijzen PN. Diaphragm dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2005; 172:200-205.
14. Yu F, Degens H, Li X, Larsson L. Gender- and age-related differences in the regulatory influence of thyroid hormone on the contractility and myosin composition of single rat soleus muscle fibres. Pflugers Arch 1998; 437:21-30.
15. Larsson L, Li X, Yu F, Degens H. Age-related changes in contractile properties and expression of myosin isoforms in single skeletal muscle cells. Muscle Nerve Suppl 1997; 5:S74-78.
16. Harridge SD, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C, Esbjornsson M, Saltin B. Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. Pflugers Arch 1996; 432:913-920.
17. Widrick JJ, Trappe SW, Costill DL, Fitts RH. Force-velocity and force-power properties of single muscle fibers from elite master runners and sedentary men. Am J Physiol 1996; 271:C676-683.
18. Degens H, Yu F, Li X, Larsson L. Effects of age and gender on shortening velocity and myosin isoforms in single rat muscle fibres. Acta Physiol Scand 1998; 163:33-40.
19. Edman KA. The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. J Physiol 1979; 291:143-159.
20. Moss RL. Sarcomere length-tension relations of frog skinned muscle fibres during calcium activation at short lengths. J Physiol 1979; 292:177-192.
21. Li X, Larsson L. Maximum shortening velocity and myosin isoforms in single muscle fibers from young and old rats. Am J Physiol 1996; 270:C352-360.
22. Larsson L, Li X, Frontera WR. Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. Am J Physiol 1997; 272:C638-649.
23. Runge M, Rittweger J, Russo CR, Schiessl H, Felsenberg D. Is muscle power output a key factor in the age-related decline in physical performance? A comparison of muscle cross section, chair-rising test

and jumping power. Clin Physiol Funct Imaging 2004; 24:335-340.
24. Hook P, Sriramoju V, Larsson L. Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. Am J Physiol Cell Physiol 2001; 280:C782-788.
25. Hook P, Li X, Sleep J, Hughes S, Larsson L. *In vitro* motility speed of slow myosin extracted from single soleus fibres from young and old rats. J Physiol 1999; 520(Pt2):463-471.
26. Ramamurthy B, Hook P, Jones AD, Larsson L. Changes in myosin structure and function in response to glycation. Faseb J 2001; 15:2415-2422.
27. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, Pinto Plata V, Cabral HJ. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004; 350:1005-1012.